Acute Hepatotoxic Effects of Mirex in the Rat

by MICHAEL W. KENDALL
Department of Anatomy
University of Mississippi
School of Medicine
Jackson, Miss. 39216

Mirex causes hepatic induction of cytochrome P-450 in the rat and proliferation of smooth endoplasmic reticulum, as well as liver and hepatocyte enlargement, and bile stasis (BAKER et al., 1972). Since the insecticide Mirex is a chlorinated polyhalogen as is the hepatotoxin carbon tetrachloride, it seems reasonable to suspect that the liver may respond to injury by Mirex, similarly, especially regarding the deposition of lipids in regular lobular zonal patterns due to intracellular damage inflicted by the respective polyhalogen (RUBENSTEIN and KANICS, 1964). Thus, CCl_4 causes lipid accumulations in centrolobular liver cells and leads to eventual necrosis and cirrhosis (WILSON and WILLIAMS, 1969). In addition, DDT and chloroform induce centrolobular liposis, necrosis, and cirrhosis (HARTROFT, 1964) and are therefore classified as cirrhogenic agents. In contrast, phosphorous and brombenzene are capable of producing periportal fatty accumulations and are also cirrhogenic agents. In a study of the fate of Mirex in rats (MEHENDALE et al., 1972), considerable tissue storage of Mirex was observed. Fat, muscle, liver, and kidneys contained 27.8, 3.20, 1.75, and 0.76 per cent of the total dose, respectively, after seven days following oral intubation of a single dose of Mirex-C^{14} (6.0 mg/kg). Liver cells commonly respond to the toxic stress by accumulation of lipid, usually in the form of triglycerides (FREIMAN, 1971). In the expectation that Mirex may produce lipid deposition in distinctive centrolobular or periportal zones when administered at the LD_{50} dose, a lipophilic stain, Sudan black, was utilized to visualize fat inclusions. This effort represents a part of my continuing independent investigation of the cellular toxicity of Mirex to non-target organisms.

Methods

The single oral lethal dose for 50% survival of Sherman strain female rats is 365 mg/kg according to GAINES and KIMBROUGH, 1970. In the present study this dosage in corn oil was administered to 50 Sherman strain female rats. Also, 50 more similar rats were fed ad libitum a total of 365 mg/kg Mirex in their diet (Purina Lab Chow) over a period of 12 days. After 12 days (288 hours), liver tissues were fixed in 10% aqueous formalin and cold Lavdowsky's solution (ethanol, formalin, acetic acid). The latter was used to obtain fixation of glycogen. Tissue fixed in Lavdowsky's was embedded in paraffin and eight micra sections

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 5 © 1974 by Springer-Verlag New York Inc.

were made. Sections were routinely stained (LILLIE, 1965) with periodic acid-Schiff's reagent with hematoxylin (PASH). The PAS positivity of material considered as glycogen was prevented by amylase and diastase hydrolysis. Such localization-analytical procedures actually prove that this PAS-positive, granular material is a polysaccharide. Numerous chemical analyses have demonstrated that glycogen is the polysaccharide within cytoplasm of hepatic cells. To demonstrate lipid, frozen sections of formalin-fixed tissue were stained with Sudan black. Use of these methods has been described in detail by WILLIAMS, 1960.

Results and Discussion

The most relevant responses to the LD_{50} dosage of Mirex are summarized in Table I and include effects of both intraperitoneal injections and feeding of Mirex-containing food.

TABLE I

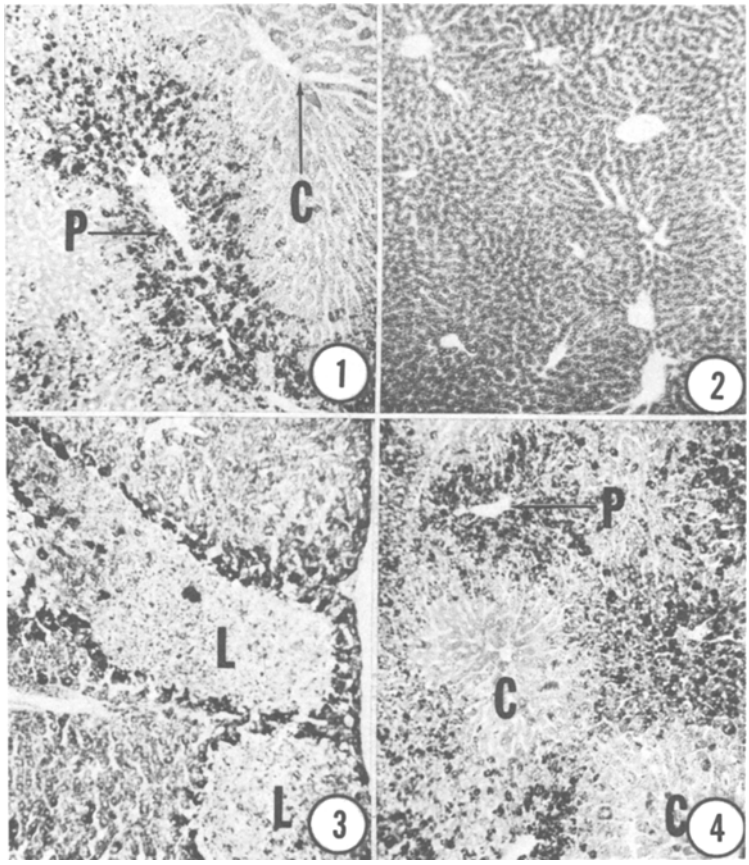
Toxicity of LD_{50} dosages of Mirex to adult female Sherman-strain rats

	Control	Intraperitoneal injection	Mirex in food
No. of rats tested	50	50	50
Day sacrificed	12th	12th	12th
No. of survivors	49	24	27
% survivors	98%	48%	54%
No. periportal liposis	0	19	23
% with periportal liposis	0%	75%	85%
No. central liposis	0	0	4
% with central liposis	0%	0%	15%
No. glycogen depletion	0	24	27
% with glycogen depletion	0%	100%	100%

Histologically, liver micro-architecture may be best comprehended by using the concept of the liver acinus first proposed by RAPPAPORT (1958). This concept which considers the liver acinus as a unit of structure and function can be utilized to best explain hepatic lobular alteration in response to toxic insult.

The single LD_{50} dosage of 365 mg/kg when administered intraperitoneally produced several distinctive histologic alterations in the liver after 12 days (288 hours). These included apparent depletion of stainable glycogen, hepatocyte enlargement, periportal liposis associated with central necrosis, and infrequent foci of necrosis on the surface (Fig. 3). When the LD_{50} dosage was fed *ad libitum* all the above types of injury were also produced except that surface lesions were absent.

In groups of rats fed a total of 365 mg/kg approximately half (46%) died before the 12th day. The remaining survivors



Figures 1-4. Hepatic tissue from rats exposed and unexposed to Mirex.

Figure 1. Liver from rat injected intraperitoneally with 365 mg/kg Mirex in corn oil solution. Note sudanophilic lipid accumulation in portal areas (P) whereas central area (C) is lipid-free.

Figure 2. Liver from control rat unexposed to Mirex. No stained fat is evident.

Figure 3. Liver from rat injected intraperitoneally with 365 mg/kg Mirex in corn oil solution. Note prominent circumscribed lesion (L) near surface of liver and projecting down into the hepatic parenchyma.

Figure 4. Liver from rat fed Mirex-containing Purina Laboratory Chow (365 mg/kg). Note periportal lipid accumulation around portal areas (P) and no lipid accumulation in central areas (C). This is similar to the pattern observed in Figure 1.

exhibited obvious lethargy, loss of hair and weight, tremor, and diarrhea which were quite similar to those symptoms and signs described by GAINES and KIMBROUGH (1970). These investigators also observed apparent glycogen depletion in rats in response to sublethal dosages of Mirex but chemical analysis for glycogen showed no decrease. Similar observations of decreased stainable glycogen were noted in this study. The diminished amount of stainable (PAS positive) glycogen present in treated rats does indicate some type of subtle biochemical alteration, however. GAINES and KIMBROUGH also noticed hepatocyte enlargement but indicate that unlike DDT, Mirex did not produce hepatic liposis. Since these investigators based their conclusion on a lower dosage (25 ppm) than was used in the present study, it seems apparent that higher dosages (365 ppm) of Mirex, like DDT, do cause lipid accumulation and this occurs in a periportal zonal pattern (Figs. 1, 3, 4). No sudanophilic positive material (lipid) is observed in liver tissue from unexposed controls (Fig. 2).

The extremely distinctive histologic pattern of periportal liposis produced by this polyhalogen may be utilized as an important aspect of Mirex toxicity in rats since very few compounds are capable of inducing the accumulation of lipid in the periportal pattern (HARTROFT, 1964). The possibility that Mirex may also be cirrhogenic is being presently investigated in this laboratory.

Summary

Mirex-induced hepatic damage included hepatocyte enlargement, glycogen depletion, focal surface necrosis, and periportal liposis when injected intraperitoneally. All the above alterations except surface lesions also occurred when Mirex was mixed with the food and fed ad libitum.

Acknowledgement

This research has been supported by the U.S. Public Health Service Grant No. 3-501-RR05386. The author would like to thank Dr. W. Lane Williams for reviewing the manuscript and offering helpful suggestions that were incorporated in this paper.

References

BAKER, R. C., L. B. COONS, R. B. MAILMAN and E. HODGSON: Environ. Res. 5, 418 (1972).

GAINES, T. B. and R. D. KIMBROUGH: Arch. Environ. Health 21, 7 (1970).

FREIMAN, D. G.: Abnormal intracellular and pericellular accumulations. In J. W. Bronson and E. A. Gall (ed.). Concepts of Disease, A Textbook of Human Pathology. New York: MacMillan Co. 1971.

HARTROFT, W. S.: Experimental hepatic injury. In L. Schiff (ed.) Diseases of the Liver. pp. 92-124. Philadelphia: Lippincott 1956.

LILLIE, R. D.: Histopathologic Technique and Practical Histochemistry. 3rd Edicition, New York: McGraw-Hill 1965.

MEHENDALE, H. M., L. FISHLEIN, M. FIELD and H. B. MATHEWS: Bull. Environ. Cont. & Tox. 8, 200 (1972).

RUBINSTEIN, D. and L. KANICS: Can. J. Biochem. Physiol. 42, 1577 (1964).

RAPPAPORT, A. M.: Anat. Rec. 130, 673 (1958).

WILLIAMS, W. L.: Yale J. Biol. Med. 33, 1 (1960).

WILSON, E. R. and W. L. WILLIAMS: Anat. Rec. 165, 391 (1969).